The role of apolipoprotein A-IV in regulating glucagon-like peptide-1 secretion

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Wang F, Yang Q, Huesman S, Xu M, Li X, Lou D, Woods SC, Marziano C, Tso P. The role of apolipoprotein A-IV in regulating glucagon-like peptide-1 secretion. Am J Physiol Gastrointest Liver Physiol 309: G680–G687, 2015. First published August 20, 2015; doi:10.1152/ajpgi.00075.2015.—Both glucagon-like peptide-1 (GLP-1) and apolipoprotein A-IV (apoA-IV) are produced from the gut and enhance postprandial insulin secretion. This study investigated whether apoA-IV regulates nutrient-induced GLP-1 secretion and whether apoA-IV knockout causes compensatory GLP-1 release. Using lymph-fistula mice, we first determined lymphatic GLP-1 secretion by administering apoA-IV before an intraduodenal Ensure infusion. apoA-IV changed neither basal nor Ensure-induced GLP-1 secretion relative to saline administration. We then assessed GLP-1 in apoA-IV−/− and wild-type (WT) mice administered intraduodenal Ensure. apoA-IV−/− mice had comparable lymph flow, lymphatic triglyceride, glucose, and protein outputs as WT mice. Intriguingly, apoA-IV−/− mice had higher lymphatic GLP-1 concentration and output than WT mice 30 min after Ensure administration. Increased GLP-1 was also observed in plasma of apoA-IV−/− mice at 30 min. apoA-IV−/− mice had comparable total gut GLP-1 content relative to WT mice under fasting, but a lower GLP-1 content 30 min after Ensure administration, suggesting that more GLP-1 was secreted. Moreover, an injection of apoA-IV protein did not reverse the increased GLP-1 secretion in apoA-IV−/− mice. Finally, we assessed gene expression of GLUT-2 and the lipid receptors, including G protein-coupled receptor (GPR) 40, GPR119, and GPR120 in intestinal segments. GLUT-2, GPR40 and GPR120 mRNAs were unaltered by apoA-IV knockout. However, ileal GPR119 mRNA was significantly increased in apoA-IV−/− mice. GPR119 colocalizes with GLP-1 in ileum and stimulates GLP-1 secretion by sensing oleic acid, lysophosphatidylcholine, and 2-monoglycerides. We suggest that increased ileal GPR119 is a potential mechanism by which GLP-1 secretion is enhanced in apoA-IV−/− mice.

apoA-IV; GLP-1; enteroinsular axis; GPR119; glucose homeostasis

Apolipoprotein A-IV (apoA-IV) is a 46-kDa apolipoprotein produced mainly by the small intestine (19). In addition to its role in lipid binding (7) and chylomicron formation (24), apoA-IV also influences food intake and glucose homeostasis. Both central and peripheral administration of apoA-IV reduce food intake (20, 21, 23). Culnan and colleagues (5) found that plasma apoA-IV is significantly increased in humans after gastric bypass surgery, coincident with the amelioration of reduced food intake and metabolic complications. Population studies by Visvikis et al. (37) and Larson et al. (17) found that the human apoA-IV polymorphism G360H is associated with elevated fasting glucose. Such reports collectively imply that apoA-IV is an integral component of the gut-brain regulation of metabolism. To identify the role of apoA-IV in the long-term control of food intake and glucose homeostasis, we backcrossed apoA-IV knockout (KO) mice onto the C57BL/6J background for over 10 generations (15), since that strain is widely used for metabolic research. Intriguingly, although apoA-IV KO mice had normal food intake and body weight, they had fasting hyperglycemia when chronically maintained on a high-fat diet, and impaired glucose-stimulated insulin secretion (38). We also found that apoA-IV stimulates insulin secretion from pancreatic islets under conditions of high circulating glucose (38), suggesting that apoA-IV comprises an important component of the enteroinsular axis; i.e., the collective signaling pathways between the gut and the pancreatic islets that control nutrient-dependent insulin secretion.

Glucagon-like peptide-1 (GLP-1), which is secreted by intestinal L cells, is also a major component of the enteroinsular axis. GLP-1 stimulates insulin secretion, and acute antagonism of GLP-1 diminishes insulin secretion and increases blood glucose (13, 16). The observation that both apoA-IV and GLP-1 are synthesized in the small bowel and secreted during and after meals has thus stimulated considerable interest in the physiology of these peptides and their potential therapeutic use for glucose control, and GLP-1 mimetics have been developed as anti-diabetic reagents by pharmaceutical companies (2).

It is unknown whether apoA-IV and GLP-1 interact during macronutrient absorption. We previously found that, when apoA-IV is injected intraperitoneally (IP), it is rapidly transported into the lymph draining the intestines (22), thus supporting the notion that even large molecules (apoA-IV: 43,000 molecular weight), when introduced into the peritoneal cavity, can be rapidly absorbed by the gut and transported into lymph. Moreover, IP injection of apoA-IV also yielded a higher lymphatic concentration at 30 min and a much longer period of elevated apoA-IV peptide than did intravenous administration (22). We also found that a single IP injection of 1 μg/g apoA-IV results in a peak plasma apoA-IV concentration at 2 h following the injection and significantly improves glucose tolerance in apoA-IV−/− animals (38). The point is that, when administered IP, apoA-IV enters both the plasma and the lymph and likely the intestinal wall as well. It is, therefore, positioned to influence the secretion and/or action of GLP-1. In the present study, we tested the hypothesis that IP apoA-IV changes the secretion of GLP-1 that occurs in response to an intraduodenal infusion of Ensure.

The enteroinsular axis accounts for ~50% of postprandial insulin release (27). It is, therefore, not surprising that several signals from the gut share similar functions in regulating insulin secretion and glucose control. Furthermore, it has been...
proposed that disruption of one component of the enteroinsular axis may be compensated for by enhancement of another (30, 31). We, therefore, also tested the hypothesis that apoA-IV KO mice, relative to wild-type controls, have enhanced secretion of GLP-1 to compensate for the loss of apoA-IV function.

Traditionally, the in vivo study of the incretin hormones involves the measurement of circulating levels in the peripheral blood and occasionally in hepatic-portal blood. However, due to the large volume of blood flow, the concentrations of the incretin hormones are usually quite low in plasma, and interpretation is often clouded by the rapid degradation of incretins by plasma enzymes (2). This renders the study of GLP-1 secretion in mice difficult not only due to their small size, but also due to the sensitivity of the assay and the volume of blood required. For instance, with the GLP-1 ELISA assay, a minimum of 100 μl of plasma are required. Because of the high lymphatic GLP-1 concentration, a much smaller volume of lymph can be used for the determination of GLP-1 secretion. We, therefore, used lymph-fistula mice, since the levels of lymphatic incretins are relatively high and the rate of degradation is lower than in plasma (1, 6, 14, 25, 29, 39).

MATERIALS AND METHODS

Animals. Adult male wild-type (WT) C57BL/6J and apoA-IV−/− mice were maintained on a 12:12-h light-dark cycle with lights on at 06:00. Animals had free access to food and water, except where noted. All procedures were approved by the University of Cincinnati Internal Animal Care and Use Committee and complied with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Lymphatic duct cannulation. Mice were fasted overnight and anesthetized with ketamine (80 mg/kg) and xylazine (20 mg/kg). A laparotomy was performed, and the superior mesenteric lymph duct was cannulated with a polyvinylchloride (PVC) tube (0.2 mm inner diameter, 0.5 mm outer diameter; Tyco Electronics, Castle Hill, NSW, Australia), which was fixed in place with a drop of cyanoacrylate glue (Krazy Glue, New York, NY) and externalized through a stab wound in the right flank. A second PVC tube (for administering nutrients; 0.5 mm inner diameter, 0.8 mm outer diameter; Tyco Electronics) was placed into the stomach through the fundus and threaded into the duodenum for Ensure infusion. The fundal opening was closed by a purse-string suture and sealed with a drop of cyanoacrylate glue. A third PVC cannula (0.5 mm inner diameter, 0.8 mm outer diameter; Tyco Electronics) was placed in the abdomen for apoA-IV or saline injection. After surgery, mice received a 5% glucose in saline infusion (0.3 ml/h) via the duodenal cannula to compensate for fluid and electrolyte loss due to lymphatic drainage. Mice recovered overnight in Bollman restraint cages housed in a temperature-regulated chamber maintained at 30°C.

Experiment I. Administration of a mixed liquid meal and lymph sampling in normal mice. Overnight-fasted C57BL/6J mice received 1 μg/g body wt recombinant apoA-IV protein (38) or saline through the IP cannula 2 h before receiving a bolus of (0.3 ml, 0.44 kcal) Ensure Original Nutrition Shake (Abbott, IL). Lymph was collected hourly from 2 hours before until 6 hours after the intraduodenal Ensure (0.3 ml, 0.44 kcal). GLP-1, triglyceride, glucose, and protein concentrations were determined as described above.

Experiment II. Administration of ensure and lymph sampling in normal and apoA-IV−/− mice. Lymph was collected from fasted mice from 1 h before until 6 h after the intraduodenal Ensure (0.3 ml, 0.44 kcal). GLP-1, triglyceride, glucose, and protein concentrations were determined as described above.

Experiment III. Determination of GLP-1 content in intestinal segments and plasma. Small intestinal segments (duodenum, jejunum, and ileum) and plasma were collected from overnight-fasted WT and apoA-IV−/− mice, as well as from mice 30 min after receiving an intraduodenal bolus of 0.3 ml Ensure. For plasma GLP-1 quantification, mice were rapidly anesthetized by IP injection of ketamine (80 mg/kg) and xylazine (20 mg/kg) and decapitated, and trunk blood samples were collected in EDTA tubes containing 1% DPP-4 inhibitor (DPP4-010, Millipore, MO). The blood samples were centrifuged for 10 min at 8,000 rpm, 4°C, and the plasma was collected for active GLP-1 measurement by ELISA. Following death, the entire small intestine was isolated, washed with phosphate-buffered saline, and sectioned into duodenum (4 cm below the pylorus), jejunum (~15 cm), and ileum (10 cm above the cecum), as described (35). The tissue was homogenized in acid-ethanol containing 74% ethanol with 0.15 M HCl (5 ml/g tissue) for the determination of GLP-1 concentrations. The homogenized tissues were extracted overnight at 4°C and centrifuged at 12,000 g for 10 min. The supernatants were then diluted 50-fold with phosphate-buffered saline containing 1 mg/ml bovine serum albumin, and active GLP-1 levels in the supernatants were determined by ELISA.

Experiment IV. The effect of apoA-IV injection on GLP-1 secretion in apoA-IV−/− mice. Overnight-fasted apoA-IV−/− mice received 1 μg/g body wt recombinant apoA-IV protein or saline through the IP cannula 2 h before receiving the bolus of Ensure. Lymph samples were collected as described in experiment I. Active GLP-1 levels were measured by ELISA.

Experiment V. Real-time PCR. Duodenal, jejunal, and ileal samples were collected from overnight-fasted WT and apoA-IV−/− mice for gene expression measurement. We collected 3 samples (0.5 cm in length, proximal end, middle, distal end) from each segment of the intestine. All three samples were used for RNA preparation. Total RNA was extracted using RNasy isolation Kit (74104, QIAGEN) and converted into complementary DNA (cDNA) using iScript (170–1880, Bio-Rad). The complementary DNA from these samples were pooled for quantitative PCR analysis. Quantitative real-time PCR was performed on a Bio-Rad iCycler system. The following primers were used: G protein-coupled receptor (GPR) 40, forward GGCCTCATTACCTCGCCCGG-3’; reverse GGGACGGCTTCTCTCATGCGG-3’; GPR 119, forward GGCAAGCAGGACCTTTCTCTACATG; reverse GATGATGCTGGTGTGCTGACGT-3’; GPR 120, forward GGTGCCGGAGCTGTCATGTG; reverse GCAAGAATGGGGCGGAAGTTCAG-3’; GPR 153, forward GGCGAGCAGGAGCTTCTCTCTTCT; reverse GCCAATGAGGATCGG-3’. Statistics. All data are presented as means ± SE. Statistical analyses were performed using GraphPad Prism 6.0. Secretion studies with time course were analyzed by two-way ANOVA, followed by Bonferroni’s posttest. Other measurements were analyzed by two-tailed Student’s t-test. A difference was considered significant if P < 0.05.

RESULTS

apoA-IV protein does not alter GLP-1 secretion. We determined the lymphatic GLP-1 concentration and output after a single IP injection of 1 μg/g of apoA-IV protein 2 h before a bolus of intraduodenal Ensure (0.3 ml, 0.44 kcal). The lymph flow rates in mice treated with apoA-IV vs. saline are depicted in Fig. 1A. In the fasting state, the average rate of lymph flow in saline-treated mice was 0.21 ml/h and was not significantly decreased from basal to 1 h after injection.
Fig. 1. A bolus injection of apolipoprotein A-IV (apoA-IV) has no effect on lymph flow. A: lymph was collected from wild-type (WT) mice treated with either saline \((n = 4)\) or \(1 \mu g/g\) apoA-IV \((n = 4)\) for 2 h pre- and 6 h post-Ensure infusion, as described in MATERIALS AND METHODS. Lymph flow rate was defined as the volume of lymph collected per hour. B: cumulative lymph flow was measured in the 2 h pre- and 6 h post-Ensure infusion. Values are means ± SE.

different from that of mice treated with apoA-IV \((0.20 \text{ ml/h})\). In saline-treated mice, lymph flow increased to a peak rate of \(0.32 \text{ ml/h}\) at 30 min after Ensure infusion; and the lymph flow rate in apoA-IV-treated rats increased to a peak of \(0.38 \text{ ml/h}\) at 30 min post-Ensure infusion. For both groups, lymph flow rates gradually declined to basal levels by 180 min post-Ensure infusion. There was no significant difference in the cumulative outputs of lymph in the 2 h pre- or 6 h post-Ensure infusion between the two groups (Fig. 1B). The important point is that, whereas intraduodenal Ensure increased lymphatic flow rate, the administration of apoA-IV had no effect in and of itself.

Fasting lymphatic GLP-1 was comparable for the two groups over the 2 h before Ensure infusion (Fig. 2A). After Ensure infusion, GLP-1 concentration increased dramatically within 15 min in both groups, peaking at \(91.54 ± 26.01 \text{ pM}\) in apoA-IV-treated mice and \(93.26 ± 25.71 \text{ pM}\) in saline control mice. Lymphatic GLP-1 concentration began to decrease in both groups by 1 h post-Ensure infusion. At 2 and 3 h, apoA-IV-treated mice tended to have higher lymphatic GLP-1 concentration than saline control mice, but the difference was not statistically significant. At later time points of 4–6 h, GLP-1 concentrations were not different between the two groups (Fig. 2A). Therefore, the administration of apoA-IV had no effect on lymphatic flow or GLP-1 levels (and presumably secretion rate) to the administration of intraduodenal Ensure.

Based on the lymph flow rates, we calculated the total lymphatic GLP-1 output during the entire 2 h pre- and 6 h post-Ensure infusion. As expected, neither hourly outputs (Fig. 2B) nor total outputs (Fig. 2C) of GLP-1 differed between apoA-IV-treated and saline-control mice. Moreover, triglycer-
apoA-IV KO has no effect on intestinal lymph flow, or on lymphatic transport of nutrients induced by Ensure. For both apoA-IV−/− and WT mice, fasting lymph flow rates were around 0.2 ml/h, peaked at comparable levels 30 min following Ensure infusion, and gradually declined back to basal levels by 120 min (Fig. 3A). Also, the cumulative lymph outputs for the 6-h study were comparable between the two groups (Fig. 3B). We then determined the effect of apoA-IV KO on lymphatic triglyceride, glucose, and protein outputs. As depicted in Fig. 4A, for both groups, lymphatic triglyceride concentration increased gradually after Ensure infusion and returned to basal level after 6 h, with no significant difference at any time point studied. Hourly outputs of triglyceride tended to be higher at 1 h and lower at 3 h in apoA-IV−/− mice than in WT mice, but the differences never reached statistical significance (Fig. 4B), such that the total output of 6-h triglyceride was comparable between the two groups (Fig. 4C). As depicted in Fig. 4D, glucose concentrations tended to be lower in apoA-IV−/− mice than in WT mice from 1 h; however, when calculated by hourly output (Fig. 4E) and total output (Fig. 4F), there were no differences between the two groups. Lymphatic protein concentrations (Fig. 4G) and outputs (Fig. 4H and I) after Ensure infusion were not significantly different between the WT and apoA-IV−/− mice.

apoA-IV−/− mice have increased lymphatic GLP-1 in response to ensure. As depicted in Fig. 5A, fasting levels of lymphatic GLP-1 were comparable in apoA-IV−/− (37.31 ± 6.14 pM) and WT mice (35.96 ± 5.70 pM). Lymphatic GLP-1 concentration and output increased markedly between 15 min and 30 min after intraduodenal Ensure. In apoA-IV−/− mice, the peak of 108.44 ± 12.52 pM occurred at 30 min, an increase of 2.9-fold over baseline. In WT mice, the peak concentration of GLP-1 was only 75.44 ± 9.58 pM at 30 min, or 2.1-fold
above baseline, and was significantly lower than the peak in apoA-IV−/− mice (P < 0.05). Differences in hourly outputs of GLP-1 in plasma were measured after overnight fasting or 30 min after an intraduodenal bolus of Ensure (n = 4–8). D: GLP-1 content in intestinal segments was measured after overnight fasting (n = 4). F: GLP-1 content in intestinal segments was determined 30 min after an intraduodenal bolus of Ensure (n = 4). Values are means ± SE. *P < 0.05, ***P < 0.001 between WT and apoA-IV−/− mice at the same time point.

apoA-IV protein administration does not alter GLP-1 secretion in apoA-IV−/− mice. We determined the lymph flow rate and lymphatic GLP-1 concentration after a single IP injection of 1 μg/g of apoA-IV protein 2 h before a bolus of intraduodenal Ensure in apoA-IV−/− mice. In the fasting state, the average lymph flow was in the range of 0.1–0.2 ml/h and was not significantly different between the two groups. Lymph flow increased to comparable peak rates at 30 min after Ensure infusion in the two groups and declined to basal levels by 2 h post-Ensure infusion (Fig. 6A). Fasting GLP-1 was comparable in the two groups over the 2 h before Ensure infusion (Fig. 6B). After Ensure infusion, GLP-1 concentration increased dramatically within 15 min in both groups, peaking at 30 min. But GLP-1 concentrations were not different between the two groups at any time (Fig. 6B).

apoA-IV KO results in increased GPR119 expression in ileum. mRNA levels of GPR40, GPR119, GPR120, and GLUT-2 were comparable in the duodenum of apoA-IV−/−
and WT mice (Fig. 7A). Likewise, in jejunum, GPR40, GPR119, GPR120, and GLUT gene expression were not significantly different between apoA-IV−/− and WT mice, although GPR119 had a trend to be increased in apoA-IV−/− mice (by 2.7-fold) relative to WT mice (P < 0.05), whereas GPR40, GPR120, and GLUT-2 were unaltered (Fig. 7C).

**DISCUSSION**

Our laboratory previously reported that apoA-IV−/− mice are glucose intolerant on a chow diet, which is caused by an attenuated insulin response to the rise of circulating glucose (38). Glucose intolerance in apoA-IV−/− mice can be corrected by a single IP administration of 1 g/g recombinant mouse apoA-IV. The same dose of apoA-IV is also sufficient to promote insulin secretion during a glucose tolerance test in animals fed a high-fat diet (38). In the present study, we used an intraduodenal Ensure bolus as a nutrient challenge. Ensure reflects complete, balanced nutrition with a calorie distribution of 22% fat, 64% carbohydrate, and 14% protein; furthermore, administering nutrients intraduodenally minimizes the effect of variability in the rate of gastric emptying. We found that an IP injection of 1 g/g apoA-IV stimulated GLP-1 secretion neither during fasting, nor upon the challenge of Ensure infusion (Fig. 2). These data indicate that the acute effect of apoA-IV on improving glucose clearance may not involve increased GLP-1 secretion. We, therefore, speculate that apoA-IV does not rely on GLP-1 to regulate glucose homeostasis. This is consistent with our laboratory’s previous finding that apoA-IV itself directly stimulates insulin secretion from isolated pancreatic islets (38).

The results presented here clearly demonstrate that lymphatic GLP-1 output (Fig. 5) is enhanced in apoA-IV−/− mice in response to an Ensure challenge, whereas general gut permeability and transport of nutrients are intact and remain unchanged (Fig. 4). GLP-1 is secreted from enteroendocrine L cells that are in direct contact with the gut lumen (8) and are, therefore, positioned to sense the chemical composition of the nutrients in the intestinal lumen. The finding that Ensure-stimulated secretion of GLP-1 is higher in apoA-IV−/− animals than in WT animals suggests that, in the absence of circulating apoA-IV, the response of GLP-1 to nutrients may be enhanced. However, when apoA-IV protein was acutely injected back to the KO mice, it did not reverse the increased GLP-1 secretion (Fig. 6). These data suggest that long-term absence of apoA-IV may cause a chronic adaptation of L cells in response to nutrient stimulation. It is not known at this point whether a continuous chronic apoA-IV injection that mimics the endogenous apoA-IV secretion may block the compensatory response of GLP-1.

Ensure contains protein, carbohydrate, and fat, all of which are stimuli of GLP-1 release in vivo. However, carbohydrate and lipids are more potent stimuli than proteins (40, 41). It was reported that GLUT-2 KO mice have impaired GLP-1 secretion, and that blocking GLUT-2 in the gut abolishes GLP-1 secretion (3, 26), indicating that GLUT-2 is important in mediating GLP-1 secretion. In apoA-IV−/− mice, GLUT-2 expression in duodenum, jejunum, and ileum were not changed compared with levels in WT controls (Fig. 7), supporting the

![Fig. 6. A bolus injection of apoA-IV has no effect on lymph flow rate and lymphatic GLP-1 concentration in apoA-IV−/− mice. A: lymph was collected from apoA-IV−/− mice treated with either saline (n = 4) or 1 μg/g apoA-IV (n = 4) for 2 h pre- and 6 h post-Ensure infusion. B: lymphatic GLP-1 concentration was measured for 2 h pre- and 6 h post-Ensure infusion.](http://www.ajpgi.org/)
idea that increased GLP-1 secretion in apoA-IV−/− mice is unlikely to arise from stimulation of L cells by luminal sugars through upregulation of GLUT-2. Numerous signaling mechanisms have been proposed to explain how L cells might sense glucose, including ATP-sensitive potassium channel closure (28), sodium glucose cotransporter activity (10), and activation of local sweet taste receptors (33). We do not have sufficient data to define the relative importance of the various pathways to stimulate GLP-1 secretion in apoA-IV−/− mice.

An intriguing finding was that the expression of GPR119, a G protein-coupled receptor, doubled in jejunum and almost tripled in the ileum of apoA-IV−/− mice relative to levels in WT controls (Fig. 7). The density of L cells increases along the length of the gastrointestinal tract, with the highest numbers being found in the ileum and colon (8). GPR119 is expressed by L cells and colocalized with GLP-1 (4). The natural ligands for this receptor are oleoylethanolamide and lysophosphatidyl-choline which signal through GPR119 and lead to increased GLP-1 secretion from L cells (4, 18). A recent report suggests that 2-oleoyl glycerol is also a GPR119 agonist and stimulates GLP-1 release (11). Although how apoA-IV KO increases GPR119 expression remains unclear, the upregulation of GPR119 may play an important role in the enhanced GLP-1 secretion from apoA-IV−/− mice. Furthermore, increased GPR119 expression likely explains why apoA-IV−/− mice have enhanced GLP-1 secretion on a high-fat diet (36).

The higher GLP-1 concentration at 30 min after Ensure infusion was also observed in plasma (Fig. 5D), but the absolute level of plasma GLP-1 was much less than the level in lymph, as we have seen previously (6). In our laboratory’s previous study, we did not observe a significant difference between apoA-IV−/− and WT mice in plasma GLP-1 when we collected plasma samples in the morning, a time when rodents are usually not eating (38), suggesting that differences of GLP-1 secretion between apoA-IV−/− and WT become exaggerated by enteral nutrient stimulation. It should be noted that the route of nutrient administration in the present study is different from that in the previous study. In the present study, enteral factors that could interact with apoA-IV in mediating the insulin response would have been stimulated, whereas in the previous study (38), direct IP delivery of glucose bypassed such enteral factors; thus there would be little reason to expect a GLP-1 compensatory response playing a role.

An intriguing question for future research is whether there is an analogous upregulation of circulating apoA-IV in GLP-1 receptor KO animals. This possibility is feasible and certainly testable for future studies. GLP-1 receptor KO animals are glucose intolerant, but the defect in glucose tolerance is smaller than anticipated (9, 34). Furthermore, whereas GLP-1 receptor and gastric inhibitory polypeptide receptor double KO mice might be expected to have a real difficulty coping with regulating their blood sugar since the two major incretins are rendered useless, yet these animals still manage to regulate their blood sugar during a glucose tolerance test at a level better than anticipated (32). We hypothesize that the reason for the apparent lack of a more profound glucose homeostasis phenotype is due to compensation by enhanced apoA-IV. This research obviously is beyond the scope of the present research but certainly warrants further investigation.

In conclusion, the present experiments demonstrate that knock out of apoA-IV results in transiently enhanced lymphatic and plasma GLP-1 levels. Taken together with the finding of increased fatty acid receptor GPR119 expression in the ileum, these findings provide new evidence for plasticity in the enteroinsular axis. Future studies addressing the mechanisms underlying compensatory changes in GLP-1 secretion and its action may help us better understand the factors important for the physiological regulation of the enteroinsular axis and glucose homeostasis.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

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